CARIBOO FOREST REGION RESEARCH SECTION

Extension Note

"Making Research Work for You"

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Cariboo Forest Region, BC

USING *HYPHOLOMA FASCICULARE* INOCULATION AS A MEANS TO CONTROL ARMILLARIA ROOT DISEASE ON CALCAREOUS SOILS: TRIAL ESTABLISHMENT AND MONITORING[1]

- **The study results show that inoculating stumps on calcareous soils with the competitive fungus *Hypholoma fasciculare* is possible.**
- **Two years after inoculation, the fungus continues to grow and shows promise for mitigating timber losses due to Armillaria root disease.**

INTRODUCTION

Armillaria root disease is present throughout much of the forested landbase in the central and southeastern parts of British Columbia. In the Nelson Forest Region, within the Invermere Forest District alone, Armillaria root disease is considered to be affecting forest productivity on 22 000 ha of the operational landbase; much of this area is on calcareous soils. For more information about calcareous soils, see Kishchuk et al. 1999. The only operational treatment currently used to control Armillaria root disease is the removal of tree roots after timber harvesting.

Recent research shows that mechanical root removal techniques, when used on calcareous soils, have the side effect of spreading considerable calcareous material (Quesnel and Curran 1999). Based on what is known about the relationship between soil calcareousness and soil productivity, there is good reason to suspect that treatments that increase calcareousness might decrease soil productivity (Quesnel and Curran 1999).

As part of the Invermere Enhanced Forest Management Pilot Project (EFMPP)[2], the BC Ministry of Forests (BCMOF) has been investigating the use of a competitive fungus, *Hypholoma fasciculare*, to reduce timber losses related to Armillaria root disease. It is speculated that *Hypholoma fasciculare* will reduce Armillaria levels by outcompeting Armillaria. In laboratory experiments, *Hypholoma fasciculare* captured substrate more quickly than Armillaria, grew over top of Armillaria and through it, and appeared to exude chemicals that kill or weaken the Armillaria (see Chapman and Xiao 2000 for more details). These laboratory results are now being tested in the field.

The competitive fungus approach does not create any soil disturbance; if the technique is effective, it might have particular application on calcareous soils. An important

[1] This document has also been published as Extension Note EN-052 by the Research Section of the BCMOF's Nelson Forest Region.
[2] The goal of the Invermere EFMPP is to develop operational forest management strategies that focus on the timing, location, and intensity of management practices; the application of various harvesting systems; and forest inventory and research activities. Emphasis is placed on the principles of adaptive management.

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question with regard to the competitive fungus approach is simply whether or not the fungus will grow well in calcareous conditions. In addition, the theory behind the competitive fungus approach is that after a natural stand-replacing event, such as a fire, there is a burst of activity by saprophytes as the large input of fresh woody material decays. It is thought that the competition between saprophytes and root rot fungi after a normal stand-destroying event is what reduces the vigor of root rot fungi. To test this theory, and to see if there might be differences in populations of saprophytic fungi in different soils, a fungus survey has been established in conjunction with competitive fungus trials.

The fungus survey focuses on cord-producing fungi because they tend to be mobile in soil and would be the type of fungi that would first occupy the roots on stumps. Cord fungi make root-like structures to explore the soil and to transport nutrients and moisture from one location to another. These fungi can be identified based on cord morphology, which makes them easier to work with than many other types of fungi.

This portion of the Invermere EFMPP is a cooperative effort of the Research Section of the Nelson Forest Region, the Invermere Forest District, and the Research Section of the Cariboo Forest Region. The ultimate goals of the project are to increase forest productivity through better management of Armillaria root disease, find alternatives to the potentially soil-productivity-reducing practice of mechanical root removal on calcareous soils, and reduce costs by lessening the need for the costly practice of mechanical root removal.

The main objectives of the competitive fungus survey are:

1. Determine if a fungus competitive with *Armillaria ostoyae* can be inoculated into stumps on calcareous soils.
2. Determine if inoculation with a competitive fungus will reduce the incidence of Armillaria root disease.
3. Determine if there are differences in the populations of cord fungi in areas where Armillaria root disease is a problem versus those areas where it is not a problem.

TRIAL DESIGN AND METHODS

Several competitive fungus trials have been established in the Invermere area.

Four of the trials were established in cooperation with Crestbrook Forest Industries (now Tembec). Two of the trials are located in upper Dry Creek at different elevations (IDFdm2 and MSdk1), and two trials are located along the Kootenay River (IDFdm2). Other trials have been established in the Nelson Forest Region in conjunction with other research work going on at Whitetail Brook (IDFdm2) and Nine Mile Creek (MSdk1), and one trial has been located in a Small Business Forest Enterprise Project (SBFEP) block at Lemon Creek (IDFdm2). All trials are controlled and replicated, and some of the trials also include root removal as another treatment.

In the competitive fungus trials, stumps were inoculated with *Hypholoma fasciculare* by burying a ~1.3-kg piece of inoculum adjacent to each stump (Figure 1), just under the forest floor and adjacent to a large root and the base of the bole. Inoculum is heavy and bulky; horses were an effective means to distribute the inoculum throughout the plots (Figure 2).

For the fungus survey part of the study, pine stakes, ~45 cm long and ~8-10 cm in diameter, were used as "traps" to lure fungi so that when the stakes are pulled, the fungi occupying them can be described. Most of the stakes were installed in fall 1998, with some additional stakes added in fall 1999. Using line intersect sampling, the "traplines" were established by pounding 100 stakes into the ground at 2-m intervals. The stakes were pounded into the mineral soil to a depth of approximately 15 cm, and usually at least 30 cm of the stake remained above the ground so the stake could be found again later and pulled up.

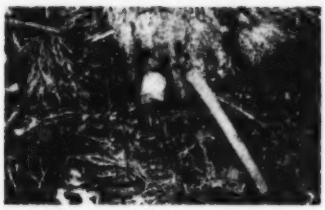

Figure 1. Inoculum was buried at the base of the bole.

Figure 2. Horse carrying inoculum.

"Traplines" were placed in a variety of scenarios including clearcuts, clearcuts with stumping, partial cuts, and uncut forested areas, both with and without Armillaria root disease—all on calcareous soil. Plots were also established in non-calcareous clearcuts, clearcuts with stumping, Armillaria-infected uncut forests, and uninfected uncut forests. In addition, stakes will be placed in the Long-Term Soil Productivity (LTSP) trial which is being established in the Mud Creek Area.[3]

The proof of whether or not the treatment with *Hypholoma fasciculare* will mitigate timber losses from Armillaria root disease will not be evident for several years. Seedling deaths caused by contact with rhizomorphs of Armillaria stumps typically do not start appearing until four or five years after harvesting (Morrison et al 1991). Four years after distributing the inoculum, seedlings and trees closest to the 100 stumps located in the core of each plot will be monitored for signs and symptoms of Armillaria

[3] The LTSP is an international soil research trial; one replicate of the trial has been installed at the Mud Creek site under the auspices of the Invermere EFMPP, and two more are under way, to make a complete installation of three replicates.

infection, including seedling mortality.

So far it has been practical to monitor the stumps to see if the competitive fungus is established. This is done by using an excavator to pull stumps, or by digging up individual roots. A certain amount can be discerned by peeling the bark away from around the stump and the upper roots. In some cases, the Hypholoma produces fruiting bodies (mushrooms). However, it is not possible to predict when the fruiting bodies will emerge, so to find them, the visit to the site has to be fortuitously timed. Hypholoma growing on wood can be identified in the field by its pattern of rot, abundant whitish cords, and distinctive smell.

In both 1998 and 1999, several stumps from the Upper Dry Creek installations were examined to determine if they had become infected with Hypholoma fasciculare.

Also, in the fall of 1999, forty trap stakes were removed, ten each from four different treatments: the partial cut area with high Armillaria root disease and not inoculated with Hypholoma; same as above except inoculated with Hypholoma; an uncut forest with high Armillaria root rot; and a clearcut in which there was no sign of Armillaria root disease.

The stakes were packaged individually in clean bags and taken back to the laboratory where they were kept refrigerated until cord fungi on the trap stakes were described and cultured. The reason for taking stakes from only a few treatments at this time was to work out the methodology—to be sure that the fungi could be described and cultured from cords. A larger sample will be taken in 2000.

RESULTS

Inoculation Evaluation

Hypholoma mushrooms were found growing on a few stumps in 1998 (Figure 3). All of the root systems excavated in 1998 had Hypholoma cords running over the surface of the root systems, and the surface wood was infected by the fungus. The identity of the Hypholoma was confirmed by cultures taken from the cords and from the surface of the wood in the root. The root systems were from 10 to >50% occupied by the Hypholoma.

In 1999, four stumps, heavily infected with Armillaria and inoculated with Hypholoma, were excavated. Hypholoma was still found on all four stumps, ranging from a relatively small patch near the point of inoculation, to being widespread over the root system (especially on pine). At the slightly higher elevation installation at Dry Creek, abundant Hypholoma was found, and in one case Hypholoma fruiting bodies were found on the side of a stump. Hypholoma seemed more vigorous in the higher elevation site.

Fungus Trapping

Many of the stakes examined had a variety of cord fungi on them, including Hypholoma (Figure 4). The sample size is too small to say anything definitive about treatment effects, but the stakes from the clearcut had fewer types of fungi on them. *Armillaria* species have also been isolated from the stakes; based on the appearance of the branching pattern of the rhizomorphs, the fungus could be *Armillaria ostoyae*.

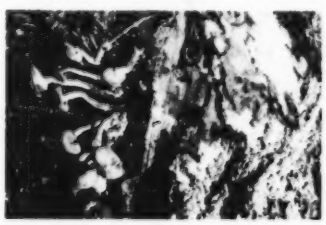

Figure 3. Hypholoma growing from an inoculated stump.

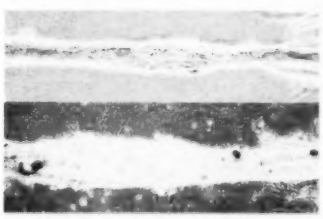

Figure 4. Two different kinds of cord fungi found at the Dry Creek study sites (unidentified cords from trap stakes).

DISCUSSION

So far, the study has clearly shown that it is possible to inoculate a competitive fungus onto stumps on calcareous soils.

The fungus is continuing to grow two years after inoculation, and shows promise for mitigating timber losses due to Armillaria root disease. Even though the fungus has established wherever it was inoculated, there seem to be differences in tree species preference and site adaptation of isolates. More stump excavations will be done in 2000 and 2001, to elaborate on how the inoculum behaves in different conditions.

The fungus-trapping technique looks as if it will be useful for monitoring populations of cord fungi. Additional stakes will be sampled from a number of treatment replicates in in 2000 and 2001.

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